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Review

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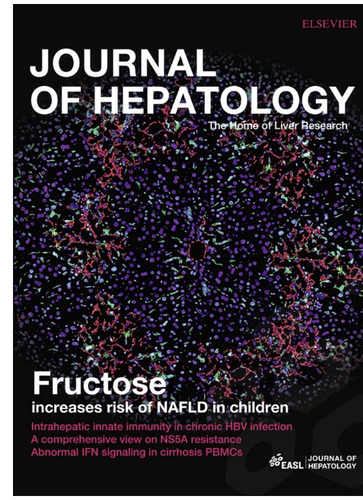

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Mesenchymal stromal cell therapy for liver diseases

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Summary

The therapeutic potential of mesenchymal stromal cells (MSC) in the treatment of liver fibrosis is predominantly based on their immunosuppressive properties, and their ability to secrete various trophic factors. This potential has been investigated in clinical and pre-clinical studies. Although the therapeutic mechanisms of MSC transplantation are still not fully characterized, accumulating evidence has revealed that various trophic factors secreted by MSC play key therapeutic roles in regeneration by alleviating inflammation, apoptosis, and fibrosis as well as stimulating angiogenesis and tissue regeneration in damaged liver. In this review, we summarize the safety, efficacy, potential transplantation routes and therapeutic effects of MSC in patients with liver fibrosis. We also discuss some of the key strategies to enhance the functionality of MSC, which include sorting and/or priming with factors such as cytokines, and also genetic engineering of MSC.

Introduction

Liver disease is a major cause of mortality and morbidity that is rising globally [1, 2]. There remain many inflammatory liver conditions for which treatments are not effective and often such patients will progress to end-stage liver disease and require liver transplantation. To prevent progression to end-stage liver disease and also to treat those with advanced fibrosis mesenchymal stromal cell (MSC) therapies have been considered and shown to have potential in such liver diseases [3-5].

MSC have been shown to have beneficial effects in a range of clinical settings including heart failure [6], lung injury [7, 8], graft versus host diseases [9] and stroke [10], as well as being reported to ameliorate liver injury in the setting of both acute and chronic liver damage [11, 12]. The pleiotropic effects of MSC represent a potential advantage over pharmacological therapies and principally focus on their ability to modulate different components of the immune system either directly or by the release of paracrine factors. In addition to these immunomodulatory effects, MSC have been shown to reduce liver injury by ameliorating oxidative stress through release of antioxidants [12] and also through anti-fibrotic effects [3, 5]. In addition, MSC have been reported to have an ability to differentiate to hepatocyte-like cells which may show promise in augmenting liver regeneration [13, 14]. These encouraging pre-clinical data have resulted in many clinical trials [15, 16], and it is therefore timely to review the data underpinning these effects and also address the important remaining scientific questions so as to establish MSC therapy for patients with liver disease.

MSC: Definition, Biology and Tissue Origins

MSC were initially described in the 1968 by Friedenstein [17], and are a subtype of adult fibroblast-like cells that have the capacity of self-renewal with high proliferative ability. They can undergo tri-lineage differentiation both *in vivo* and *in vitro* down connective tissue lineages to become osteoblasts, chondrocytes and adipocytes.

MSC are plastic adherent cells originally identified and isolated from bone marrow but due to their limited number (0.01 to 0.001% of total bone marrow cells) [18] and invasive nature of their isolation from bone marrow, researchers explored alternative sources. Several studies have reported the successful isolation of MSC from different tissues with similar *in vitro* properties, including synovial membrane [19], adipose tissue (AT) [20], umbilical cord blood (UCB) [21], amniotic fluid (AF) [22] and placenta [23]. Umbilical cord tissue (UC) has been a particularly promising source of MSC - cells can be isolated from several compartments within UC including umbilical vein, umbilical arteries, umbilical cord perivascular tissue, Wharton's jelly (WJ) and sub-amniotic tissue. Furthermore, MSC isolated from UC tissue are believed to be more primitive than other cells isolated from other tissues and are found in higher numbers, ensuring this source is gaining prominence. Notably, MSCs from different sources display similar expression profile of MSCs surface markers and morphological features in culture, yet they have different levels of tri-lineage differentiation potential [24]. In addition, further differences have been related to the culture conditions, especially in the isolation procedure and culturing protocols, as well as the experiment protocol used [25]. Whereas, direct comparisons of MSCs from different sources have been shown to share similar biological properties [26-28], other authors demonstrated differences in immunomodulatory properties between BM-MSCs, UC-MSCs, and AT-MSCs [29, 30]. In addition, umbilical cord MSCs exhibit a higher proliferative capacity in comparison to MSCs populations obtained from other sources [24].

MSC from differing sources such as AT, UCB, and BM were found to express a similar pattern of surface antigens [31], although there was variation in respect to their differentiation potential, morphology and proliferation rate [32]. Moreover, several studies have demonstrated that BM MSC have higher expression of pluripotency genes such as Oct-4, Nanog, and Sox-2 than those isolated from WJ and AT [33].

Potential mechanisms of action of MSC in liver disease

Mechanism of immunomodulation by MSC

MSC can modulate and repair injured tissue by modulating injurious immune responses through a range of mechanisms including direct cell to cell interaction or remotely by secretion of paracrine factors (Fig. 1) [34]. Of note, MSC have reduced immunogenicity due to a lack expression of class II major histocompatibility (MHC) antigens when unprimed and do not express many of the molecules required for immune recognition such as CD80, CD86n and CD40 [35].

Immunomodulatory effect of MSC on adaptive immunity

MSC can inhibit the proliferation of T cells *in vitro* by either secretion of soluble factors or by direct interaction with T-lymphocytes (Fig. 2) [36]. Several different molecules secreted by MSC have been reported to have an immunomodulatory effect on T-cell activities, including transforming growth factor β (TGF-β), hepatocyte growth factor (HGF) [36], prostaglandin E2 (PGE2) [37], and indoleamine 2,3-dioxygenase (IDO) [38]. Notably, the production of these immunomodulatory molecules differs according to the source of MSC, for example, WJ-MSC produce higher amounts of TGF-β than BM-MSC [39].

The inflammatory environment is known to have an essential role during the interaction between MSC and T cells, for example, the immunosuppressive capacity of MSC is induced by treatment with combination of cytokines (IFN-γ, IL-1α, TNF-α, and IL-1β) [40]. These cytokines can enhance some chemokines and other immune cells to easily contact the MSC and mediate the immune reactions. Another mechanism by which MSC can suppress the proliferation of T cells is via secretion of nitric oxide (NO) which causes inhibition of STAT5 pathways [41]. Another study demonstrated that MSC can secrete matrix metalloproteinases (MMP), such as MMP-2 and MMP-9, which suppress T cell activation by cleaving surface CD25 from T cells [42].

MSC have also been shown to promote the generation and development of regulatory T cells (Tregs), which can positively influence balance of immune damage during tissue injury [43]. The induction of CD4+ CD25+ FOXP3+ Treg was mediated by secretion of TGF-β [44] and is accompanied by an inhibition of the proliferation and differentiation of Th1 and Th17 helper T cells which can further trigger activation of regulatory T cells. This mechanism was associated with an increased production of IL-10 by MSC [45].

MSC can also inhibit the proliferation of B cells, and reduce their production of immunoglobulin. Glennie *et al* used CD40 and IL-4 to increase the proliferation rate of murine B cells and demonstrated that subsequent co-culture with MSC significantly inhibited their proliferation [46]. In addition, MSC resulted in a significant stimulation in immunoglobulin production after co-culture of B cells in trans-well experiment [47]. MSC may also alter surface expression of chemokine receptors on B cells; co-culture with MSC in 1:1 ratio resulted in a significant reduction of expression of CXCR4, CCR7 and CXCR5 on B cells [48]. CXCR4 was found to significantly reduced even with 1:10 ratio when cultured with MSC, suggesting that MSC can specifically target CXCR4 which has a role in homing and fate of MSC [49].

Natural killer cells (NK) represent a critical component of the immune response against viral infections and tumor cells [50] - Sotiropoulou *et al* demonstrated that MSC reduced IL-15 secretion from IL-2 induced NK cells. This reduction was presumed to be due to either cell-to-cell interaction or release of soluble factors such as PGE2 and TGF-β [35]. In addition, another group reported that MSC can suppress NK cells after stimulation with IL-5 [50]. In models of acute liver injury MSC ameliorated hepatotoxicity of NKT cell in an indoleamine 2,3-dioxygenase (IDO) dependent manner, by reducing the number of IL-17 cells and stimulation of FOXP3 and IL-10 resulting from increased numbers of NK Treg in the injured liver [51].

Immunomodulatory effect of MSC in innate immunity

Macrophages can be classified into classical pro-inflammatory macrophages (M1) or alternative macrophages (M2) that secret anti-inflammatory cytokines (Fig. 2) [52]. MSC have been reported to trigger polarization of M1 toward M2 both *in vivo* and *in vitro*. This polarization is driven by the ability of MSC to secrete soluble factors such as interleukin (IL)-

10 and IL-1Ra which have been shown to attenuate liver injury by promoting number of M2 macrophages [53]. In addition to the IL-10 mediated ability of MSC to promote switching phenotype of macrophages from M1 to M2, MSC can also help to promote survival of monocytes through upregulation of CCL18, which was found to indirectly mediate ability of MSCs to induce Tregs formation [44] as demonstrated in animal models of sepsis and colitis [54]. In this study murine adipose derived MSC significantly increased the proportion of M2 like cells by increased production of IL10 and arginase1 activities [54].

MSC can also regulate, and interact with, dendritic cell function (DC) by blocking differentiation of antigen presenting cells (APC) to monocytes and decreasing their expression of anti-inflammatory molecules such as IL12, TNF-α, and IFN-γ, whilst also enhancing their secretion of IL-10 which may induce regulatory T cell numbers (Fig. 2) [55]. Notably, WJ-MSC can also inhibit the differentiation of monocytes to mature dendritic cells when cultured with CD14+ monocytes, indicating an indirect effect of WJ-MSC on the allogeneic response of T cells [56]. There is now therefore a greater recognition of the importance of the microenvironment on the immunomodulatory capacity of MSC [40], prompting a need for a better understanding of the microenvironment associated with specific diseases so as to develop more effective therapeutic efficacy of MSC.

Anti-fibrotic activities of MSC

Inflammation and fibrosis have a very close relationship in liver disease. In response to liver injury, pro-fibrotic factors such as TGF-β, platelet-derived growth factor (PDGF), IL-13 and IL-4, which are secreted by resident or infiltrating immune cells, play important roles in the activation and proliferation of hepatic stellate cells (HSC), which are important cells for the production of ECM in the liver [57-60]. Therefore, the anti-fibrotic activities of MSC can be distinguish the direct or indirect effects on HSC. The indirect anti-fibrotic effects on HSC are achieved by MSC controlling immune cells and sequentially inhibiting the activity of HSC, whereas the direct anti-fibrosis effects on HSC are mediated by MSC inhibiting the activity of HSC.

As the indirect anti-fibrotic effects of MSC on HSC, MSC can regulate the activities of HSC by modulating immune cell activity mentioned above. MSC can migrate towards injured sites of inflammatory reaction where they are exposed to inflammatory cytokines such as IFN-γ and IL-1β [61, 62]. These MSC secrete various soluble mediators (e.g. NO, PGE2, IDO, IL-6, IL-10, and HLA-G), thus resulting in the suppression of the proliferation and activation of a variety of immune cells as well as the induction of Treg cells [63]. Thus, suppression of immune cell activities by MSC can also reduce fibrogenic processes and ameliorate ECM accumulation in liver disease. In particular, macrophages play a central role in both fibrosis and fibrotic resolution in the liver [64, 65] - during hepatic fibrogenesis, pro-inflammatory M1 macrophages located near the activated hepatic myofibroblasts secrete pro-fibrogenic factors such as TGF-β, PDGF, and CCL2. This secretion leads to increased fibrogenic responses of the myofibroblasts through the promotion of their activation, proliferation, and chemotaxis [64, 66]. However, macrophages co-cultured with MSC are polarized into anti-inflammatory M2 states, which show higher phagocytic activity through increased expression of IL-10 and decreased expression of tumor necrosis factor (TNF)-α and IL-12p40 [67, 68]. These results suggest that MSC can induce changes in the cytokine profile of activated macrophages promoting resolution of fibrosis. PGE2 has also been reported as a major immunomodulatory molecule when MSC are co-cultured with macrophages [69-71].

As the direct anti-fibrotic effects of MSC on HSC, MSC can inhibit proliferation and ECM production potential of HSC and also induce apoptosis of HSC. MSC can secrete IL-10, HGF, TGF-β3 and TNF-α, inhibit the proliferation of HSC, and decrease ECM synthesis [72, 73]. TGF-β3 and HGF induce G0/G1 cell cycle arrest of HSC by upregulating $p21^{Cip1}$ and $p27^{Kip1}$ and downregulating cyclin D1, which leads to HSC growth inhibition [73]. Similarly, neutralization of secretion of TNF-α and IL-10 from MSC inhibits activated HSC proliferation and ECM synthesis [72]. Moreover, MSC-derived HGF can also accelerate the rate of HSC apoptosis [72] and MSC cultured with HGF improve serum albumin level and reduce liver fibrosis in rats [74]. The Notch pathway is activated during direct co-culture of MSC and HSC through a cell–cell contact mode and results in significant suppression of the proliferation and α-SMA expression of HSC [75]. In liver fibrosis, activated HSC can express the tissue inhibitors of metalloproteinase (TIMP)-1 and TIMP-2, specific inhibitors of MMP [76], whereas MSC have been reported to increase the expression of MMP (e.g. MMP-2, -9, -13 and -14) [77-79] or decrease TIMP-1 expression [80], which are generally associated with fibrosis resolution in experimental models.

Hepatocyte-like differentiation of MSC

Since hepatocytes have been reported to improve liver function and mitigate fibrosis in preclinical and clinical studies, hepatocyte transplantation has been considered an alternative therapy to replace liver transplantation. Several factors influence the hepatic differentiation of MSC. It has been reported that the treatment of MSC with a combination of several growth factors, cytokine, and chemical compounds (i.e., HGF, fibroblast growth factor [FGF]-2/-4, epidermal growth factor [EGF], oncostatin M [OSM], leukemia inhibitory factor [LIF], dexamethasone [Dex], insulin-transferrin-selenium [ITS], and/or nicotinamide [NTA]) increases the expression of hepatocyte markers such as HNF-3β, GATA4, CK19, transthyretin, α-fetoprotein, albumin, and CK18 [81]. In addition, when MSC are co-cultured with liver cells [82] or grown by pellet culture [83], they can be differentiated into hepatocyte-like cells. The differentiation of MSC into hepatocytes has been reported in rats [84], mice [85], sheep [86] and humans [87]. Moreover, hepatic stem/progenitor cells isolated from the adult human liver have been reported to be much better at being able to differentiate into hepatocytes when compared with MSC isolated from other tissues than liver [88]. Several groups have also reported that MSC differentiated into hepatocytes can help improve liver function and histopathologic grade, although they are less effective than adult hepatocytes [89]. There still remains uncertainty in the literature about the characterisation of MSC-derived hepatocytes which requires further evaluation, and indeed it is unclear if this will be a major means by which MSC are utilised.

Clinical trials using MSC in liver disease

Many clinical studies have been conducted on the treatment of liver disease using MSC, focusing on clinical trial design, cell sources, injection route, patient groups, and efficacy of therapies [15, 16, 90-104]. Based on these viewpoints, we addressed 17 articles to summarize MSC-based therapy for liver disease from 2007 to July 15, 2017 (Table 1). With regard to study design for the treatment of liver disease using MSC, there was one case series, six case control studies, five cohort studies, and five randomized clinical trials (RCTs) (Table 1). Cohort and case control studies have been performed in the early clinical trials [15, 16, 90-92, 94, 96-100, 104], and RCT studies seem to be mainly conducted recently to evaluate the efficacy of MSC [93, 95, 101-103]. In the reported studies, a marked heterogeneity was found in injected cell dosage, stem cell source, graft type, injection route, and study design, but significant adverse effects were not reported in the included studies. The diseases of the patients included acute-on-chronic liver failure (ACLF), liver failure including cirrhosis due to alcohol, HBV, or HCV, and primary biliary cholangitis. A total of 688 patients were enrolled in the clinical studies, with a range of four patients in the case series design [94] to 158 patients in the case control design [99]. In the clinical studies [99], BM-derived MSC (BM-MSC) were used in 14 studies and UC-MSC were used in the remaining three studies. In five studies allogenic MSC were used to treat liver disease; two were derived from the BM, and three were from UC [15, 16, 96, 100, 102]. Moreover, autologous BM-derived hepatocytes were reported to improve Child-Pugh score, MELD score, fatigue scale, and performance status over the controls, although no comparison was made with any undifferentiated MSC transplantation groups [97]. However, in recent animal studies, it has been reported that undifferentiated MSC can more effectively improve liver function than MSC differentiated into hepatocytes [89]. Jang et al. analyzed the liver function improvement after repeated MSC injections at 4 and 8 weeks [91]. In pilot studies, hepatic fibrosis was found to be ameliorated or reduced in six of 11 patients (54.5%) and the Child score improved in ten patients (90.9%) [91]. However, in the inter-group comparison (one-time injection versus two-time injection), two-time BM-MSC transplantation was not found to improve fibrosis over a single transplantation [101]. When three studies using two injection routes were analyzed separately [92, 97, 104], the peripheral vein (PV) was found to be most commonly used as a transplantation route in 11 cases; the hepatic artery (HA) was used in four cases, intra-splenic (IS) injection was used in three cases, intrahepatic (IH) injection in one case, and portal vein in one case. There was no difference in the efficacy of MSC based on the route of administration (PV, IS, portal vein or IH) [92, 97, 104] and in the incidence of HCC or mortality in hepatic failure patients with hepatitis B between the autologous MSC-infused and the control groups [99]. In an efficacy analysis after MSC transplantation, 15 studies reported benefits of using MSC, but two did not._

Taken together, the results of all these studies can be summarized to say that MSC treatments for patients with liver disease are safe and may improve liver function, although robust randomised clinical studies are required to gain confidence with regard to the clinical efficacy of MSC. However, to improve the efficacy of MSC therapy for liver disease, pre-clinical and clinical studies are necessary to standardize the best delivery route of MSC, to optimize the sufficient number of MSC, and to elongate the survival duration of engrafted MSC. Furthermore, in order to understand the therapeutic mechanism and fate of MSC more clearly, it is required to develop a specific biomarker with low toxicity so that the transplanted MSC can be accurately tracked.

Future perspectives

Whilst conventional unmanipulated MSC have been the mainstay of therapeutic studies thus far there have been extensive efforts to try and enhance their efficacy. This section will review some of the key strategies which include sorting MSC to enrich for greater functionality, priming of MSC with factors such as cytokines and finally genetic engineering of cells (Fig. 3). The main driver for these approaches is to enhance efficacy and/or organ homing although there is also often a need to create/protect intellectual property so as to generate a viable business model. The challenge therefore is to balance the additional costs and potential logistical/safety concerns associated with such perturbations against improvements in efficacy.

MSC enrichment

MSC represent heterogeneous populations of cells, therefore, sorting approaches are highly considered to achieve homogenous populations of MSC, resulting in enriched subsets which could crucially produce various selected populations with different therapeutic functions and open new strategies for the modification of MSC for more beneficial effects.

MSC are phenotypically diverse both morphologically and functionally and thus sorting cells based on marker expression may allow for the selection of cells with greater efficacy. This does require definition of which function is being focused on, and often markers of stemness or proliferation are reported, whereas immunomodulatory action may be the most important.

Sorting of cells for pre-clinical studies is relatively straightforward and can use a range of modalities including flow cell sorting which should result in high purity yields. It is more challenging however when such approaches are attempted in clinical practice as they need to adhere more closely to good manufacturing practice (GMP) which can restrict the modality used. Clinically approved modalities such as the CliniMACS are clinically accredited but may not result in high purities of rare populations and thus the use of GMP fluorescence cell sorting analysis is encouraging.

$CD146^{+}$ is expressed on various cells types including endothelia cells [24] and can contribute to biological functions such as cell migration, proliferation and differentiation [105, 106]. CD146 expression is correlated with cellular senescence of MSC and markedly affects the proliferation, differentiation, and stemness of hUCB-MSC. Sorted $CD146^+$ MSC have delayed cellular senescence which is mediated by regulation of Bmi-1, id1, and Twist1 expression, which can regulate the cellular senescence process [107]. This suggested that CD146+ could be a novel marker responsible for control of senescence of MSCs and hence improve the therapeutic efficacy of MSCs.

In a recent study, sorting MSC sub-populations based on $CD73^+$ expression has demonstrated greater self-renewal and differentiation properties [108]. These sorted cells ($CD73^+$) exhibited high levels of colony forming unit ability in contrast with an absence observed with $CD73^-$ cells._

Another study has characterized populations of MSC using several markers, including $CD271^+$, known as nerve growth factor receptor and proposed as a marker of BM stromal cells, adhesion molecule (CD56), and MSCA-1^+ (mesenchymal stem cell antigen-1) [109]. Sorted dual-positive MSCA-1^+ and $CD56^+$ MSC were reported to have 2-4 greater clonal efficiency than MSCA-1^+ $CD56^-$. However, MSCA-1^+ $CD56^-$ were shown to have potential ability to differentiate into adipocytes, whereas MSCA-1^+ $CD56^+$ were restricted to chondrogenic and pancreatic like cells differentiation. Similarly, other reports indicate that enrichment of synovium-derived-MSC using CD271 in combination with THY-1 (CD90) results in greater chondrogenic differentiation ability and colony forming potential in the CFU-F assay compared to $CD271^+$ $CD90^+$ BM-MSC. Thus, this combination could be a good candidate for the isolation of MSC from different tissue sources for cartilage regeneration [110].

Sherman et al. [111] have proposed aldehyde dehydrogenase (ALDH) as a marker for MSC which defines an enhanced ability to contribute to revascularization. MSC isolated from human bone marrow and purified into ALDH^{hi} and ALDH^{lo} populations had identical expression of MSC surface makers and ability to differentiate into adipocytes, osteoblasts, and chondroblasts *in vitro*. Notably though conditioned medium from ALDH^{hi} MSC was shown to promote endothelial cell expansion *in vitro* and enhance recruitment of endogenous vascular cells after subcutaneously implanted in NOD/SCID mice, which was mediated by up-regulation of lectin [111].

Positive selection on the basis of expression of the Stro-1 specific marker has also been proposed and such MSC are enriched with respect to CFU-F progenitors [112]. Stro-1^{+} expanded MSC were reported to have better migratory capacity in various tissues when compare to Stro-1^{-} [113]. Other research groups were able to increase expression of cytokines related cardiovascular which can be mediated through using Stro-1^{+} enriched MSC [114].

Expression of CD200 has also been used to purify MSC [115], with its expression inhibiting osteoclast formation via inhibition of RANKL signalling pathways, which consequently reduce expression of osteoclast associated genes such as tartrate resistance acid phosphatase (TRAP) and nuclear factor of activated T cells cytoplasmic 1 (NFARC1) [116]. Another study has clearly shown that CD200^{+} BM-MSC can modulate the immune response of macrophages by inhibition of TNF-α secretion when compared to CD200^{low} BM-MSC [117]. Consisting with its role in immunomodulation, MSC have been identified to drive the expression of CD200 in T cell subsets following co-culture with MSC [118]. This upregulation was reported in both CD4^{+} and CD8^{+} T lymphocyte.

More recently, CD362^{+} (Syndecan-2) marker has been identified as a novel marker to select a homogeneous population of MSC with enhanced immunomodulatory properties (patent number WO 20131177661 A1). This marker has recently investigated for its ability to reduce immunogenicity and enhance the immunomodulatory ability in liver inflammation [119, 120]. Syndecan-2 found to be expressed in hematopoietic cells and myeloid cells [121]. And functionally reported to upregulate upon T cell activation and play significance role in CD3 downregulation through degradation of T-cell receptor (TCR) [122]. These findings strongly suggest that enrichment of syndecan-2 expression in MSC could play an essential role in immune modulation in injured tissue.

The potential benefits of the various markers that have been used to select/enrich MSC are detailed in Table 2.

MSC priming

As with selection of MSC, priming of cells before use is intended to enhance their biological properties for whichever clinical indication is being considered (Table 3). This may include improvements in MSC immunomodulatory effects, homing to injured organs and/or greater expansion of cells.

Enhancing immunomodulatory properties of MSC

Pre-treatment of MSC with the pro-inflammatory cytokines IL-1β, IL-23 and IL-6 for 96 hours [123] was found to enhance secretion of TGF-β and reduce production of IL-4 by MSC, although notably no changes were reported in production of IFN-γ and TNF-α. In addition, cytokine-treated MSC exhibited superior multi-lineage differentiation capacity compared to untreated MSC, with no associated changes in their morphology. IL-1 appears to be important for pre-conditioning of MSC, as combined treatment with IL-1α and IL-1β increases production of granulocyte-colony stimulating factor (G-CSF) and secretion of anti-inflammatory mediators such as IL-10. Moreover, microglial cells incubated with conditioned medium from IL-1 primed MSC increase expression of anti-inflammatory cytokines such as IL10 and decrease secretion of pro-inflammatory cytokines as reported in TNF-α and IL-6 [124].

Duijvestein et al. [125] showed that stimulation MSC with IFN-γ enhanced the anti-inflammatory response of MCS in experiment colitis animal model. In addition, IFN-γ primed MSC exhibit a significant reduction in TNF-α and IL-6 in colon homogenates, while normal MSC had no effect. In the same model, activation of MSC with IFN-γ further promote the immunomodulation via enhance production of IL-17 and IL-4, which therefore inhibit the Th1 and reduce T cell activation [125]. Under similar conditions, pre-stimulation of BM-MSC with IFN-γ and TNF-α stimulate production of IL-6, HGF, TGF-β [126]. More interestingly, an in vivo GVHD model, administration of MSC pre-treated with IFN-γ have the capability to enhance survival rates of mice with GVHD, resulted in 100% survival [127].

More recently, data from de Witte and colleagues have demonstrated that pre-treatment of UC-MSC with different treatments such as TGF-β, IFN-γ, IFN-β or in combinations (TGF-β, IFN-γ and retinoic acid) suppress expression of CD107a on NK cells, enhancing MSC immunomodulation. In addition, MSC treated with IFN-γ and the multiple cytokine combination were found to significantly upregulate IDO activities which subsequently suppressed CD4 and CD8 proliferation when compare to untreated MSC. Notably, following infusion into mice injured with a single dose of CCl_4, a higher percentage of TGF-β treated MSC homed to the injured liver (25%) compared with untreated MSC (13%) [119].

In another liver injury studies, IL-7 treated MSC had a superior therapeutic effect on liver injury mediated in part through increased activation of iNOS. IL-17 down-regulates gene expression of ARE/poly(U)-binding/ degradation factor 1 (AUF-1) in MSC which is a protein known to regulate immune related molecules [128] and has a key role in regulation stromal cell fate [129]. Thus, AUF1 could have a novel role to enhance the effect of IL-17 on immunosuppression. Similarly, IL-17a modified MSC have been reported to suppress proliferation of T cell *in vitro* via mechanisms such as inhibition of Th1 cytokines (IFN-γ, TNF-α, IL-10, and IL-2), enhance production of IL-6 and induction of regulatory T cells [130].

IL-6 priming of MSC infused into an acute model of CCl_4 injury resulted in improved viability of isolated hepatocytes as well as a reduction in expression of pro-apoptotic markers such as BAX, Caspase-3 and LDH activities. This finding was not observed when MSC or IL-6 treatment were applied alone [131]. In addition, administration of IL-6 with MSC was found to enhance repair of liver injury in a mouse model of liver fibrosis with reductions in fibrosis, improvements in liver synthetic function, promote hepatocyte survival, and decrease apoptosis in fibrotic liver [131].

Enhancing homing of MSC

A study demonstrated that adhesion molecules such as ICAM and VCAM can be highly expressed on MSC following priming with a combination of IFN-γ, TNF-α and IL-1. This upregulation of expression of ICAM and VCAM led to increased recruiting of MSC to vascular endothelium, this close contact of MSC with immune cells could enhance the immunosuppressive properties of MSC [132, 133]. Similarly, MSC pre-treated with IFN-γ, TNF-α can induced regulatory T cells more efficiently than non-treated MSC. Furthermore, MSC pre-incubated with IFN-γ, TNF-α induced secretion of CCR6 and therefore increase the adhesion of Th17 cells to MSC, resulting in promote the generation of regulatory T cells ($FOXP3^+$ cell) from Th17 cells and consequently improve their immunosuppressive properties [134].

Priming with CXCL9 has also been shown to enhance adherence of MSC to endothelial cells as well as increase spreading of MSC on the endothelial cells as characterized by the extension of pseudopodia in multiple directions [135]. Further characterization of the beneficial effect of chemokines on MSC behaviour was reported in the same study using trans-well migration experiments, in which MSC migrated across endothelial layers in the presence of chemokines such as CXCL9, CXCL16, and CXCL20, and CXCL25. Of note no migration was observed in the presence of TNF-α alone.

Genetic modification of MSC (Gene editing)

Beside enrichment and priming MSC *in vitro*, transplantation of MSC after genetic correction or modification (gene editing) represents a powerful approach to use of MSC in regenerative medicine (Table 4). This section will review progress with genetic engineering approaches that have reported with MSC, including viral and non-viral manipulations. Viral transfection of MSC can be achieved with several approaches including lentivirus, adenovirus and retrovirus [136].

MSC have also been genetically modified to increase expression of CXCR4, thereby improving their homing to the injured liver and reducing liver damage [137]. Similarly, the same finding was reported in a rat model of lung injury, with increased expression of CXCR4 on MSC resulting in enhanced hepatic migration and improvement of their immunomodulatory properties mediated by increased production of IL-10 and reduction in TNF-α. Notably, these findings suggest that overexpression of CXCR4 not only enhanced MSC homing but also increased their immunosuppressive effects [138].

Further examination of the beneficial effects of genetic modified MSC was reported in a mouse model of liver fibrosis, following overexpression of insulin growth factor like-1 (IGF-1) [139]. After systemic administration, IGF-1 modified MSC were able to significantly reduce the degree of fibrosis, likely through the down regulation of α-SMA, TGF-β and COL1A2 in animal treated with IGF-1 MSC when compare with animal treated with normal MSC [140]. Over-expression of HGF in MSC was also found to reduce liver fibrosis, seemingly mediated by a reduction in TFG-β, platelet-derived growth factor-bb (PDGF-bb), and metalloprotease-14 (MMP-14) [141]. HGF overexpressed MSC also act on hepatic stellate cells to reduce α-SMA and desmin expression, indicating that MSC that overexpress HGF decreased both the activation and number of hepatic stellate cells more greater level than MSC. This could have therapeutic effect to prevent diseases progression and foster liver restoration.

Another reprogramming approach showed that over-expression of miR-27b in adipose tissue derived MSC resulted in reduction in a rat model of ischemic liver injury in rat with improvements in ALT, AST, TNF-α, and IL-6 as well as significance suppression in TGF-β

[142]. Moreover, these transfected cells were shown to have anti-fibrotic ability with suppression of MMP-2 and MMP-9 in liver tissue.

Further study linked between the genetic modified MSC and their capacity to express endothelial cell (EC) markers with similar function. For example, silencing MMP-2 and MMP-14 with endothelial growth medium can induce the MSC differentiation into EC by enhance production of endothelial markers, such as PECAM and VE-cadherin. These markers were increase from 4 to 15% and from 4 to 30% after silencing MMP-2 and MMP-12, respectively. This observation was in comparison with MSC that treated with endothelial growth medium only [143].

In other work, the expression level of HO-1 was genetically modified in MSC and shown to have resistance to cell death under oxidative stress condition and enhance their anti-apoptotic properties [144]. Moreover, HO-1 overexpressed MSC have shown to have more surviving cells following exposure to H_2O_2 and hypoxia, indicating that HO-1 may shape the stress responsive and cytoprotective properties of MSC. Notably, in the murine model of myocardial infarction, overexpression of HO-1 resulted in diminished oxidative stress and apoptosis as well as an enhanced effect on angiogenesis. This was associated with a 2.1 fold up-regulation of VEGF levels compared to normal MSC [145].

Conclusions and outlook

MSC therapy is generally regarded as a safe and potentially relevant therapeutic strategy for patients with chronic liver disease, including ACLF, liver failure including cirrhosis due to alcohol, HBV, or HCV, and primary biliary cholangitis. However, in order for MSC therapy to be established as a clinical therapeutics for those of liver diseases, further robust randomised clinical studies are required to increase the reliability of the clinical efficacy of MSC. In addition, further studies on optimal delivery route, sufficient number of MSC, and extension of survival of engrafted MSC are needed to enhance the efficacy of MSC therapy. However, several concerns still remain, including the low migration and fibrogenic potential of MSC, the optimal sources, and the risk of oncogenesis and viral transmission. Whilst, conventional unmanipulated MSC have constituted the mainstream of therapeutic clinical studies so far, there have been extensive efforts to enhance their efficacy, including enrichment and/or priming of MSC along with genetic engineering of cells. The main driver for these approaches is to enhance efficacy and/or organ homing although there is also often a need to create/protect intellectual property so as to generate a viable business model as well as balancing the additional costs and potential safety issues against enhanced efficacy.

References

[1] Shiels MS, Chernyavskiy P, Anderson WF, Best AF, Haozous EA, Hartge P, et al. Trends in premature mortality in the USA by sex, race, and ethnicity from 1999 to 2014: an analysis of death certificate data. Lancet 2017;389:1043-1054.

[2] Williams R, Alexander G, Aspinall R, Bosanquet J, Camps-Walsh G, Cramp M, et al. New metrics for the Lancet Standing Commission on Liver Disease in the UK. Lancet 2017;389:2053-2080.

[3] Zhang Z, Wang FS. Stem cell therapies for liver failure and cirrhosis. J Hepatol 2013;59:183-185.

[4] Forbes SJ, Newsome PN. Liver regeneration - mechanisms and models to clinical

application. Nat Rev Gastroenterol Hepatol 2016;13:473-485.

[5] Haldar D, Henderson NC, Hirschfield G, Newsome PN. Mesenchymal stromal cells and liver fibrosis: a complicated relationship. FASEB J 2016;30:3905-3928.

[6] Ji ST, Kim H, Yun J, Chung JS, Kwon SM. Promising Therapeutic Strategies for Mesenchymal Stem Cell-Based Cardiovascular Regeneration: From Cell Priming to Tissue Engineering. Stem Cells Int 2017;2017:3945403.

[7] Simonson OE, Mougiakakos D, Heldring N, Bassi G, Johansson HJ, Dalen M, et al. In Vivo Effects of Mesenchymal Stromal Cells in Two Patients With Severe Acute Respiratory Distress Syndrome. Stem Cells Transl Med 2015;4:1199-1213.

[8] Matthay MA, Pati S, Lee JW. Concise Review: Mesenchymal Stem (Stromal) Cells: Biology and Preclinical Evidence for Therapeutic Potential for Organ Dysfunction Following Trauma or Sepsis. Stem Cells 2017;35:316-324.

[9] Chen X, Wang C, Yin J, Xu J, Wei J, Zhang Y. Efficacy of Mesenchymal Stem Cell Therapy for Steroid-Refractory Acute Graft-Versus-Host Disease following Allogeneic Hematopoietic Stem Cell Transplantation: A Systematic Review and Meta-Analysis. PLoS One 2015;10:e0136991.

[10] Honmou O, Onodera R, Sasaki M, Waxman SG, Kocsis JD. Mesenchymal stem cells: therapeutic outlook for stroke. Trends Mol Med 2012;18:292-297.

[11] Volarevic V, Nurkovic J, Arsenijevic N, Stojkovic M. Concise review: Therapeutic potential of mesenchymal stem cells for the treatment of acute liver failure and cirrhosis. Stem Cells 2014;32:2818-2823.

[12] Kuo TK, Hung SP, Chuang CH, Chen CT, Shih YR, Fang SC, et al. Stem cell therapy for liver disease: parameters governing the success of using bone marrow mesenchymal stem cells. Gastroenterology 2008;134:2111-2121, 2121 e2111-2113.

[13] Wu XB, Tao R. Hepatocyte differentiation of mesenchymal stem cells. Hepatobiliary Pancreat Dis Int 2012;11:360-371.

[14] Lee KD, Kuo TK, Whang-Peng J, Chung YF, Lin CT, Chou SH, et al. In vitro hepatic differentiation of human mesenchymal stem cells. Hepatology 2004;40:1275-1284.

[15] Wang L, Li J, Liu H, Li Y, Fu J, Sun Y, et al. Pilot study of umbilical cord-derived mesenchymal stem cell transfusion in patients with primary biliary cirrhosis. J Gastroenterol Hepatol 2013;28 Suppl 1:85-92.

[16] Zhang Z, Lin H, Shi M, Xu R, Fu J, Lv J, et al. Human umbilical cord mesenchymal stem cells improve liver function and ascites in decompensated liver cirrhosis patients. J Gastroenterol Hepatol 2012;27 Suppl 2:112-120.

[17] <u>Friedenstein AJ, Petrakova KV, Kurolesova AI, Frolova GP. Heterotopic of bone marrow. Analysis of precursor cells for osteogenic and hematopoietic tissues. Transplantation 1968;6:230-247.</u>

[18] Gronthos S, Zannettino AC, Hay SJ, Shi S, Graves SE, Kortesidis A, et al. Molecular and cellular characterisation of highly purified stromal stem cells derived from human bone marrow. J Cell Sci 2003;116:1827-1835.

[19] De Bari C, Dell'Accio F, Tylzanowski P, Luyten FP. Multipotent mesenchymal stem cells from adult human synovial membrane. Arthritis Rheum 2001;44:1928-1942.

[20] Zuk PA, Zhu M, Mizuno H, Huang J, Futrell JW, Katz AJ, et al. Multilineage cells from human adipose tissue: implications for cell-based therapies. Tissue Eng 2001;7:211-228.

[21] Lee OK, Kuo TK, Chen WM, Lee KD, Hsieh SL, Chen TH. Isolation of multipotent mesenchymal stem cells from umbilical cord blood. Blood 2004;103:1669-1675.

[22] Antonucci I, Stuppia L, Kaneko Y, Yu S, Tajiri N, Bae EC, et al. Amniotic fluid as a rich source of mesenchymal stromal cells for transplantation therapy. Cell Transplant 2011;20:789-795.

[23] Fukuchi Y, Nakajima H, Sugiyama D, Hirose I, Kitamura T, Tsuji K. Human placenta-derived cells have mesenchymal stem/progenitor cell potential. Stem Cells 2004;22:649-658.

[24] Baksh D, Yao R, Tuan RS. Comparison of proliferative and multilineage differentiation potential of human mesenchymal stem cells derived from umbilical cord and bone marrow. Stem Cells 2007;25:1384-1392.

[25] Bara JJ, Richards RG, Alini M, Stoddart MJ. Concise review: Bone marrow-derived mesenchymal stem cells change phenotype following in vitro culture: implications for basic research and the clinic. Stem Cells 2014;32:1713-1723.

[26] Najar M, Raicevic G, Boufker HI, Fayyad Kazan H, De Bruyn C, Meuleman N, et al. Mesenchymal stromal cells use PGE2 to modulate activation and proliferation of lymphocyte subsets: Combined comparison of adipose tissue, Wharton's Jelly and bone marrow sources. Cell Immunol 2010;264:171-179.

[27] Yoo KH, Jang IK, Lee MW, Kim HE, Yang MS, Eom Y, et al. Comparison of immunomodulatory properties of mesenchymal stem cells derived from adult human

tissues. Cell Immunol 2009;259:150-156.

[28] Mattar P, Bieback K. Comparing the Immunomodulatory Properties of Bone Marrow, Adipose Tissue, and Birth-Associated Tissue Mesenchymal Stromal Cells. Front Immunol 2015;6:560.

[29] Melief SM, Zwaginga JJ, Fibbe WE, Roelofs H. Adipose tissue-derived multipotent stromal cells have a higher immunomodulatory capacity than their bone marrow-derived counterparts. Stem Cells Transl Med 2013;2:455-463.

[30] Chao YH, Wu HP, Wu KH, Tsai YG, Peng CT, Lin KC, et al. An increase in CD3+CD4+CD25+ regulatory T cells after administration of umbilical cord-derived mesenchymal stem cells during sepsis. PLoS One 2014;9:e110338.

[31] Wagner W, Wein F, Seckinger A, Frankhauser M, Wirkner U, Krause U, et al. Comparative characteristics of mesenchymal stem cells from human bone marrow, adipose tissue, and umbilical cord blood. Exp Hematol 2005;33:1402-1416.

[32] Kern S, Eichler H, Stoeve J, Kluter H, Bieback K. Comparative analysis of mesenchymal stem cells from bone marrow, umbilical cord blood, or adipose tissue. Stem Cells 2006;24:1294-1301.

[33] Secunda R, Vennila R, Mohanashankar AM, Rajasundari M, Jeswanth S, Surendran R. Isolation, expansion and characterisation of mesenchymal stem cells from human bone marrow, adipose tissue, umbilical cord blood and matrix: a comparative study. Cytotechnology 2015;67:793-807.

[34] Christ B, Bruckner S, Winkler S. The Therapeutic Promise of Mesenchymal Stem Cells for Liver Restoration. Trends Mol Med 2015;21:673-686.

[35] Klyushnenkova E, Mosca JD, Zernetkina V, Majumdar MK, Beggs KJ, Simonetti DW, et al. T cell responses to allogeneic human mesenchymal stem cells: immunogenicity, tolerance, and suppression. J Biomed Sci 2005;12:47-57.

[36] Di Nicola M, Carlo-Stella C, Magni M, Milanesi M, Longoni PD, Matteucci P, et al. Human bone marrow stromal cells suppress T-lymphocyte proliferation induced by cellular or nonspecific mitogenic stimuli. Blood 2002;99:3838-3843.

[37] Aggarwal S, Pittenger MF. Human mesenchymal stem cells modulate allogeneic immune cell responses. Blood 2005;105:1815-1822.

[38] Meisel R, Zibert A, Laryea M, Gobel U, Daubener W, Dilloo D. Human bone marrow stromal cells inhibit allogeneic T-cell responses by indoleamine 2,3-dioxygenase-mediated tryptophan degradation. Blood 2004;103:4619-4621.

[39] Prasanna SJ, Gopalakrishnan D, Shankar SR, Vasandan AB. Pro-inflammatory cytokines, IFNgamma and TNFalpha, influence immune properties of human bone marrow and Wharton jelly mesenchymal stem cells differentially. PLoS One 2010;5:e9016.

[40] Ren G, Zhang L, Zhao X, Xu G, Zhang Y, Roberts AI, et al. Mesenchymal stem cell-mediated immunosuppression occurs via concerted action of chemokines and nitric oxide. Cell Stem Cell 2008;2:141-150.

[41] Sato K, Ozaki K, Oh I, Meguro A, Hatanaka K, Nagai T, et al. Nitric oxide plays a critical role in suppression of T-cell proliferation by mesenchymal stem cells. Blood 2007;109:228-234.

[42] Ding Y, Xu D, Feng G, Bushell A, Muschel RJ, Wood KJ. Mesenchymal stem cells

prevent the rejection of fully allogenic islet grafts by the immunosuppressive activity of matrix metalloproteinase-2 and -9. Diabetes 2009;58:1797-1806.

[43] Prevosto C, Zancolli M, Canevali P, Zocchi MR, Poggi A. Generation of CD4+ or CD8+ regulatory T cells upon mesenchymal stem cell-lymphocyte interaction. Haematologica 2007;92:881-888.

[44] Melief SM, Schrama E, Brugman MH, Tiemessen MM, Hoogduijn MJ, Fibbe WE, et al. Multipotent stromal cells induce human regulatory T cells through a novel pathway involving skewing of monocytes toward anti-inflammatory macrophages. Stem Cells 2013;31:1980-1991.

[45] Luz-Crawford P, Kurte M, Bravo-Alegria J, Contreras R, Nova-Lamperti E, Tejedor G, et al. Mesenchymal stem cells generate a CD4+CD25+Foxp3+ regulatory T cell population during the differentiation process of Th1 and Th17 cells. Stem Cell Res Ther 2013;4:65.

[46] Glennie S, Soeiro I, Dyson PJ, Lam EW, Dazzi F. Bone marrow mesenchymal stem cells induce division arrest anergy of activated T cells. Blood 2005;105:2821-2827.

[47] Rasmusson I, Le Blanc K, Sundberg B, Ringden O. Mesenchymal stem cells stimulate antibody secretion in human B cells. Scand J Immunol 2007;65:336-343.

[48] Corcione A, Benvenuto F, Ferretti E, Giunti D, Cappiello V, Cazzanti F, et al. Human mesenchymal stem cells modulate B-cell functions. Blood 2006;107:367-372.

[49] Nitzsche F, Muller C, Lukomska B, Jolkkonen J, Deten A, Boltze J. Concise Review: MSC Adhesion Cascade-Insights into Homing and Transendothelial Migration. Stem Cells 2017;35:1446-1460.

[50] Sotiropoulou PA, Perez SA, Gritzapis AD, Baxevanis CN, Papamichail M. Interactions between human mesenchymal stem cells and natural killer cells. Stem Cells 2006;24:74-85.

[51] Milosavljevic N, Gazdic M, Simovic Markovic B, Arsenijevic A, Nurkovic J, Dolicanin Z, et al. Mesenchymal stem cells attenuate acute liver injury by altering ratio between interleukin 17 producing and regulatory natural killer T cells. Liver Transpl 2017;23:1040-1050.

[52] Lawrence T, Natoli G. Transcriptional regulation of macrophage polarization: enabling diversity with identity. Nat Rev Immunol 2011;11:750-761.

[53] Lee KC, Lin HC, Huang YH, Hung SC. Allo-transplantation of mesenchymal stem cells attenuates hepatic injury through IL1Ra dependent macrophage switch in a mouse model of liver disease. J Hepatol 2015;63:1405-1412.

[54] Anderson P, Souza-Moreira L, Morell M, Caro M, O'Valle F, Gonzalez-Rey E, et al. Adipose-derived mesenchymal stromal cells induce immunomodulatory macrophages which protect from experimental colitis and sepsis. Gut 2013;62:1131-1141.

[55] Beyth S, Borovsky Z, Mevorach D, Liebergall M, Gazit Z, Aslan H, et al. Human mesenchymal stem cells alter antigen-presenting cell maturation and induce T-cell unresponsiveness. Blood 2005;105:2214-2219.

[56] Tipnis S, Viswanathan C, Majumdar AS. Immunosuppressive properties of human umbilical cord-derived mesenchymal stem cells: role of B7-H1 and IDO. Immunol Cell Biol 2010;88:795-806.

[57] Desmouliere A, Geinoz A, Gabbiani F, Gabbiani G. Transforming growth factor-beta 1

induces alpha-smooth muscle actin expression in granulation tissue myofibroblasts and in quiescent and growing cultured fibroblasts. J Cell Biol 1993;122:103-111.

[58] Wynn TA. Fibrotic disease and the T(H)1/T(H)2 paradigm. Nat Rev Immunol 2004;4:583-594.

[59] Kim G, Kim MY, Baik SK. Transient elastography versus hepatic venous pressure gradient for diagnosing portal hypertension: a systematic review and meta-analysis. Clin Mol Hepatol 2017;23:34-41.

[60] Kim G, Shim KY, Baik SK. Diagnostic Accuracy of Hepatic Vein Arrival Time Performed with Contrast-Enhanced Ultrasonography for Cirrhosis: A Systematic Review and Meta-Analysis. Gut Liver 2017;11:93-101.

[61] Rasmusson I. Immune modulation by mesenchymal stem cells. Exp Cell Res 2006;312:2169-2179.

[62] Eom YW, Shim KY, Baik SK. Mesenchymal stem cell therapy for liver fibrosis. Korean J Intern Med 2015;30:580-589.

[63] Sharma RR, Pollock K, Hubel A, McKenna D. Mesenchymal stem or stromal cells: a review of clinical applications and manufacturing practices. Transfusion 2014;54:1418-1437.

[64] Wynn TA, Barron L. Macrophages: master regulators of inflammation and fibrosis. Semin Liver Dis 2010;30:245-257.

[65] Kim G, Huh JH, Lee KJ, Kim MY, Shim KY, Baik SK. Relative Adrenal Insufficiency in Patients with Cirrhosis: A Systematic Review and Meta-Analysis. Dig Dis Sci

2017;62:1067-1079.

[66] Fadok VA, Bratton DL, Konowal A, Freed PW, Westcott JY, Henson PM. Macrophages that have ingested apoptotic cells in vitro inhibit proinflammatory cytokine production through autocrine/paracrine mechanisms involving TGF-beta, PGE2, and PAF. J Clin Invest 1998;101:890-898.

[67] Kim J, Hematti P. Mesenchymal stem cell-educated macrophages: a novel type of alternatively activated macrophages. Exp Hematol 2009;37:1445-1453.

[68] Maggini J, Mirkin G, Bognanni I, Holmberg J, Piazzon IM, Nepomnaschy I, et al. Mouse bone marrow-derived mesenchymal stromal cells turn activated macrophages into a regulatory-like profile. PLoS One 2010;5:e9252.

[69] Nauta AJ, Fibbe WE. Immunomodulatory properties of mesenchymal stromal cells. Blood 2007;110:3499-3506.

[70] Keating A. How do mesenchymal stromal cells suppress T cells? Cell Stem Cell 2008;2:106-108.

[71] Kang SH, Kim MY, Baik SK. Novelties in the pathophysiology and management of portal hypertension: new treatments on the horizon. Hepatol Int 2017.

[72] Parekkadan B, van Poll D, Megeed Z, Kobayashi N, Tilles AW, Berthiaume F, et al. Immunomodulation of activated hepatic stellate cells by mesenchymal stem cells. Biochem Biophys Res Commun 2007;363:247-252.

[73] Wang J, Bian C, Liao L, Zhu Y, Li J, Zeng L, et al. Inhibition of hepatic stellate cells proliferation by mesenchymal stem cells and the possible mechanisms. Hepatol Res

2009;39:1219-1228.

[74] Oyagi S, Hirose M, Kojima M, Okuyama M, Kawase M, Nakamura T, et al. Therapeutic effect of transplanting HGF-treated bone marrow mesenchymal cells into CCl4-injured rats. J Hepatol 2006;44:742-748.

[75] Chen S, Xu L, Lin N, Pan W, Hu K, Xu R. Activation of Notch1 signaling by marrow-derived mesenchymal stem cells through cell-cell contact inhibits proliferation of hepatic stellate cells. Life Sci 2011;89:975-981.

[76] Herbst H, Wege T, Milani S, Pellegrini G, Orzechowski HD, Bechstein WO, et al. Tissue inhibitor of metalloproteinase-1 and -2 RNA expression in rat and human liver fibrosis. Am J Pathol 1997;150:1647-1659.

[77] Wu Y, Huang S, Enhe J, Ma K, Yang S, Sun T, et al. Bone marrow-derived mesenchymal stem cell attenuates skin fibrosis development in mice. Int Wound J 2014;11:701-710.

[78] Higashiyama R, Inagaki Y, Hong YY, Kushida M, Nakao S, Niioka M, et al. Bone marrow-derived cells express matrix metalloproteinases and contribute to regression of liver fibrosis in mice. Hepatology 2007;45:213-222.

[79] Meier RP, Mahou R, Morel P, Meyer J, Montanari E, Muller YD, et al. Microencapsulated human mesenchymal stem cells decrease liver fibrosis in mice. J Hepatol 2015;62:634-641.

[80] Ali G, Mohsin S, Khan M, Nasir GA, Shams S, Khan SN, et al. Nitric oxide augments mesenchymal stem cell ability to repair liver fibrosis. J Transl Med 2012;10:75.

[81] Schwartz RE, Reyes M, Koodie L, Jiang Y, Blackstad M, Lund T, et al. Multipotent adult progenitor cells from bone marrow differentiate into functional hepatocyte-like cells. J Clin Invest 2002;109:1291-1302.

[82] Lange C, Bassler P, Lioznov MV, Bruns H, Kluth D, Zander AR, et al. Liver-specific gene expression in mesenchymal stem cells is induced by liver cells. World J Gastroenterol 2005;11:4497-4504.

[83] Ong SY, Dai H, Leong KW. Inducing hepatic differentiation of human mesenchymal stem cells in pellet culture. Biomaterials 2006;27:4087-4097.

[84] Shu SN, Wei L, Wang JH, Zhan YT, Chen HS, Wang Y. Hepatic differentiation capability of rat bone marrow-derived mesenchymal stem cells and hematopoietic stem cells. World J Gastroenterol 2004;10:2818-2822.

[85] Theise ND, Badve S, Saxena R, Henegariu O, Sell S, Crawford JM, et al. Derivation of hepatocytes from bone marrow cells in mice after radiation-induced myeloablation. Hepatology 2000;31:235-240.

[86] Chamberlain J, Yamagami T, Colletti E, Theise ND, Desai J, Frias A, et al. Efficient generation of human hepatocytes by the intrahepatic delivery of clonal human mesenchymal stem cells in fetal sheep. Hepatology 2007;46:1935-1945.

[87] Banas A, Teratani T, Yamamoto Y, Tokuhara M, Takeshita F, Quinn G, et al. Adipose tissue-derived mesenchymal stem cells as a source of human hepatocytes. Hepatology 2007;46:219-228.

[88] Herrera MB, Bruno S, Buttiglieri S, Tetta C, Gatti S, Deregibus MC, et al. Isolation and characterization of a stem cell population from adult human liver. Stem Cells

2006;24:2840-2850.

[89] El Baz H, Demerdash Z, Kamel M, Atta S, Salah F, Hassan S, et al. Transplant of Hepatocytes, Undifferentiated Mesenchymal Stem Cells, and In Vitro Hepatocyte-Differentiated Mesenchymal Stem Cells in a Chronic Liver Failure Experimental Model: A Comparative Study. Exp Clin Transplant 2017.

[90] Amin MA, Sabry D, Rashed LA, Aref WM, el-Ghobary MA, Farhan MS, et al. Short-term evaluation of autologous transplantation of bone marrow-derived mesenchymal stem cells in patients with cirrhosis: Egyptian study. Clin Transplant 2013;27:607-612.

[91] Jang YO, Kim YJ, Baik SK, Kim MY, Eom YW, Cho MY, et al. Histological improvement following administration of autologous bone marrow-derived mesenchymal stem cells for alcoholic cirrhosis: a pilot study. Liver Int 2014;34:33-41.

[92] Kharaziha P, Hellstrom PM, Noorinayer B, Farzaneh F, Aghajani K, Jafari F, et al. Improvement of liver function in liver cirrhosis patients after autologous mesenchymal stem cell injection: a phase I-II clinical trial. Eur J Gastroenterol Hepatol 2009;21:1199-1205.

[93] Mohamadnejad M, Alimoghaddam K, Bagheri M, Ashrafi M, Abdollahzadeh L, Akhlaghpoor S, et al. Randomized placebo-controlled trial of mesenchymal stem cell transplantation in decompensated cirrhosis. Liver Int 2013;33:1490-1496.

[94] Mohamadnejad M, Alimoghaddam K, Mohyeddin-Bonab M, Bagheri M, Bashtar M, Ghanaati H, et al. Phase 1 trial of autologous bone marrow mesenchymal stem cell transplantation in patients with decompensated liver cirrhosis. Arch Iran Med 2007;10:459-466.

[95] Salama H, Zekri AR, Medhat E, Al Alim SA, Ahmed OS, Bahnassy AA, et al. Peripheral vein infusion of autologous mesenchymal stem cells in Egyptian HCV-positive patients with end-stage liver disease. Stem Cell Res Ther 2014;5:70.

[96] Wang L, Han Q, Chen H, Wang K, Shan GL, Kong F, et al. Allogeneic bone marrow mesenchymal stem cell transplantation in patients with UDCA-resistant primary biliary cirrhosis. Stem Cells Dev 2014;23:2482-2489.

[97] Amer ME, El-Sayed SZ, El-Kheir WA, Gabr H, Gomaa AA, El-Noomani N, et al. Clinical and laboratory evaluation of patients with end-stage liver cell failure injected with bone marrow-derived hepatocyte-like cells. Eur J Gastroenterol Hepatol 2011;23:936-941.

[98] El-Ansary M, Abdel-Aziz I, Mogawer S, Abdel-Hamid S, Hammam O, Teaema S, et al. Phase II trial: undifferentiated versus differentiated autologous mesenchymal stem cells transplantation in Egyptian patients with HCV induced liver cirrhosis. Stem Cell Rev 2012;8:972-981.

[99] Peng L, Xie DY, Lin BL, Liu J, Zhu HP, Xie C, et al. Autologous bone marrow mesenchymal stem cell transplantation in liver failure patients caused by hepatitis B: short-term and long-term outcomes. Hepatology 2011;54:820-828.

[100] Shi M, Zhang Z, Xu R, Lin H, Fu J, Zou Z, et al. Human mesenchymal stem cell transfusion is safe and improves liver function in acute-on-chronic liver failure patients. Stem Cells Transl Med 2012;1:725-731.

[101] Suk KT, Yoon JH, Kim MY, Kim CW, Kim JK, Park H, et al. Transplantation with autologous bone marrow-derived mesenchymal stem cells for alcoholic cirrhosis: Phase

2 trial. Hepatology 2016;64:2185-2197.

[102] Lin BL, Chen JF, Qiu WH, Wang KW, Xie DY, Chen XY, et al. Allogeneic bone marrow-derived mesenchymal stromal cells for hepatitis B virus-related acute-on-chronic liver failure: A randomized controlled trial. Hepatology 2017;66:209-219.

[103] Lanthier N, Lin-Marq N, Rubbia-Brandt L, Clement S, Goossens N, Spahr L. Autologous bone marrow-derived cell transplantation in decompensated alcoholic liver disease: what is the impact on liver histology and gene expression patterns? Stem Cell Res Ther 2017;8:88.

[104] El-Ansary M, Mogawer S, Abdel-Aziz I, Abdel-Hamid S. Phase I Trial: Mesenchymal Stem Cells Transplantation in End Stage Liver Disease. J Am Sci 2010;6:135-144.

[105] Tsang WP, Shu Y, Kwok PL, Zhang F, Lee KK, Tang MK, et al. CD146+ human umbilical cord perivascular cells maintain stemness under hypoxia and as a cell source for skeletal regeneration. PLoS One 2013;8:e76153.

[106] Ulrich C, Abruzzese T, Maerz JK, Ruh M, Amend B, Benz K, et al. Human Placenta-Derived CD146-Positive Mesenchymal Stromal Cells Display a Distinct Osteogenic Differentiation Potential. Stem Cells Dev 2015;24:1558-1569.

[107] Jin HJ, Kwon JH, Kim M, Bae YK, Choi SJ, Oh W, et al. Downregulation of Melanoma Cell Adhesion Molecule (MCAM/CD146) Accelerates Cellular Senescence in Human Umbilical Cord Blood-Derived Mesenchymal Stem Cells. Stem Cells Transl Med 2016;5:427-439.

[108] Suto EG, Mabuchi Y, Suzuki N, Suzuki K, Ogata Y, Taguchi M, et al. Prospectively isolated mesenchymal stem/stromal cells are enriched in the CD73+ population and

exhibit efficacy after transplantation. Sci Rep 2017;7:4838.

[109] Battula VL, Treml S, Bareiss PM, Gieseke F, Roelofs H, de Zwart P, et al. Isolation of functionally distinct mesenchymal stem cell subsets using antibodies against CD56, CD271, and mesenchymal stem cell antigen-1. Haematologica 2009;94:173-184.

[110] Ogata Y, Mabuchi Y, Yoshida M, Suto EG, Suzuki N, Muneta T, et al. Purified Human Synovium Mesenchymal Stem Cells as a Good Resource for Cartilage Regeneration. PLoS One 2015;10:e0129096.

[111] Sherman SE, Kuljanin M, Cooper TT, Putman DM, Lajoie GA, Hess DA. High Aldehyde Dehydrogenase Activity Identifies a Subset of Human Mesenchymal Stromal Cells with Vascular Regenerative Potential. Stem Cells 2017;35:1542-1553.

[112] Dennis JE, Carbillet JP, Caplan AI, Charbord P. The STRO-1+ marrow cell population is multipotential. Cells Tissues Organs 2002;170:73-82.

[113] Bensidhoum M, Chapel A, Francois S, Demarquay C, Mazurier C, Fouillard L, et al. Homing of in vitro expanded Stro-1- or Stro-1+ human mesenchymal stem cells into the NOD/SCID mouse and their role in supporting human CD34 cell engraftment. Blood 2004;103:3313-3319.

[114] Psaltis PJ, Paton S, See F, Arthur A, Martin S, Itescu S, et al. Enrichment for STRO-1 expression enhances the cardiovascular paracrine activity of human bone marrow-derived mesenchymal cell populations. J Cell Physiol 2010;223:530-540.

[115] Delorme B, Ringe J, Gallay N, Le Vern Y, Kerboeuf D, Jorgensen C, et al. Specific plasma membrane protein phenotype of culture-amplified and native human bone marrow mesenchymal stem cells. Blood 2008;111:2631-2635.

[116] Varin A, Pontikoglou C, Labat E, Deschaseaux F, Sensebe L. CD200R/CD200 inhibits osteoclastogenesis: new mechanism of osteoclast control by mesenchymal stem cells in human. PLoS One 2013;8:e72831.

[117] Pietila M, Lehtonen S, Tuovinen E, Lahteenmaki K, Laitinen S, Leskela HV, et al. CD200 positive human mesenchymal stem cells suppress TNF-alpha secretion from CD200 receptor positive macrophage-like cells. PLoS One 2012;7:e31671.

[118] Najar M, Raicevic G, Jebbawi F, De Bruyn C, Meuleman N, Bron D, et al. Characterization and functionality of the CD200-CD200R system during mesenchymal stromal cell interactions with T-lymphocytes. Immunol Lett 2012;146:50-56.

[119] de Witte SFH, Merino AM, Franquesa M, Strini T, van Zoggel JAA, Korevaar SS, et al. Cytokine treatment optimises the immunotherapeutic effects of umbilical cord-derived MSC for treatment of inflammatory liver disease. Stem Cell Res Ther 2017;8:140.

[120] de Witte SF, Franquesa M, Baan CC, Hoogduijn MJ. Toward Development of iMesenchymal Stem Cells for Immunomodulatory Therapy. Front Immunol 2015;6:648.

[121] Teixe T, Nieto-Blanco P, Vilella R, Engel P, Reina M, Espel E. Syndecan-2 and -4 expressed on activated primary human CD4+ lymphocytes can regulate T cell activation. Mol Immunol 2008;45:2905-2919.

[122] Rovira-Clave X, Angulo-Ibanez M, Noguer O, Espel E, Reina M. Syndecan-2 can promote clearance of T-cell receptor/CD3 from the cell surface. Immunology 2012;137:214-225.

[123] Pourgholaminejad A, Aghdami N, Baharvand H, Moazzeni SM. The effect of pro-inflammatory cytokines on immunophenotype, differentiation capacity and

immunomodulatory functions of human mesenchymal stem cells. Cytokine 2016;85:51-60.

[124] Redondo-Castro E, Cunningham C, Miller J, Martuscelli L, Aoulad-Ali S, Rothwell NJ, et al. Interleukin-1 primes human mesenchymal stem cells towards an anti-inflammatory and pro-trophic phenotype in vitro. Stem Cell Res Ther 2017;8:79.

[125] Duijvestein M, Wildenberg ME, Welling MM, Hennink S, Molendijk I, van Zuylen VL, et al. Pretreatment with interferon-gamma enhances the therapeutic activity of mesenchymal stromal cells in animal models of colitis. Stem Cells 2011;29:1549-1558.

[126] Linero I, Chaparro O. Paracrine effect of mesenchymal stem cells derived from human adipose tissue in bone regeneration. PLoS One 2014;9:e107001.

[127] Polchert D, Sobinsky J, Douglas G, Kidd M, Moadsiri A, Reina E, et al. IFN-gamma activation of mesenchymal stem cells for treatment and prevention of graft versus host disease. Eur J Immunol 2008;38:1745-1755.

[128] Han X, Yang Q, Lin L, Xu C, Zheng C, Chen X, et al. Interleukin-17 enhances immunosuppression by mesenchymal stem cells. Cell Death Differ 2014;21:1758-1768.

[129] Chenette DM, Cadwallader AB, Antwine TL, Larkin LC, Wang J, Olwin BB, et al. Targeted mRNA Decay by RNA Binding Protein AUF1 Regulates Adult Muscle Stem Cell Fate, Promoting Skeletal Muscle Integrity. Cell Rep 2016;16:1379-1390.

[130] Sivanathan KN, Rojas-Canales DM, Hope CM, Krishnan R, Carroll RP, Gronthos S, et al. Interleukin-17A-Induced Human Mesenchymal Stem Cells Are Superior Modulators of Immunological Function. Stem Cells 2015;33:2850-2863.

[131] Nasir GA, Mohsin S, Khan M, Shams S, Ali G, Khan SN, et al. Mesenchymal stem cells and Interleukin-6 attenuate liver fibrosis in mice. J Transl Med 2013;11:78.

[132] Ren G, Zhao X, Zhang L, Zhang J, L'Huillier A, Ling W, et al. Inflammatory cytokine-induced intercellular adhesion molecule-1 and vascular cell adhesion molecule-1 in mesenchymal stem cells are critical for immunosuppression. J Immunol 2010;184:2321-2328.

[133] Ren G, Roberts AI, Shi Y. Adhesion molecules: key players in Mesenchymal stem cell-mediated immunosuppression. Cell Adh Migr 2011;5:20-22.

[134] Ghannam S, Pene J, Moquet-Torcy G, Jorgensen C, Yssel H. Mesenchymal stem cells inhibit human Th17 cell differentiation and function and induce a T regulatory cell phenotype. J Immunol 2010;185:302-312.

[135] Chamberlain G, Smith H, Rainger GE, Middleton J. Mesenchymal stem cells exhibit firm adhesion, crawling, spreading and transmigration across aortic endothelial cells: effects of chemokines and shear. PLoS One 2011;6:e25663.

[136] Park JS, Suryaprakash S, Lao YH, Leong KW. Engineering mesenchymal stem cells for regenerative medicine and drug delivery. Methods 2015;84:3-16.

[137] Ma HC, Shi XL, Ren HZ, Yuan XW, Ding YT. Targeted migration of mesenchymal stem cells modified with CXCR4 to acute failing liver improves liver regeneration. World J Gastroenterol 2014;20:14884-14894.

[138] Yang JX, Zhang N, Wang HW, Gao P, Yang QP, Wen QP. CXCR4 receptor overexpression in mesenchymal stem cells facilitates treatment of acute lung injury in rats. J Biol Chem 2015;290:1994-2006.

[139] Sobrevals L, Enguita M, Quiroga J, Prieto J, Fortes P. Insulin-Like Growth Factor I (IGF-I) Expressed from an AAV1 Vector Leads to a Complete Reversion of Liver Cirrhosis in Rats. PLoS One 2016;11:e0162955.

[140] Fiore EJ, Bayo JM, Garcia MG, Malvicini M, Lloyd R, Piccioni F, et al. Mesenchymal stromal cells engineered to produce IGF-I by recombinant adenovirus ameliorate liver fibrosis in mice. Stem Cells Dev 2015;24:791-801.

[141] Kim MD, Kim SS, Cha HY, Jang SH, Chang DY, Kim W, et al. Therapeutic effect of hepatocyte growth factor-secreting mesenchymal stem cells in a rat model of liver fibrosis. Exp Mol Med 2014;46:e110.

[142] Chen KD, Huang KT, Lin CC, Weng WT, Hsu LW, Goto S, et al. MicroRNA-27b Enhances the Hepatic Regenerative Properties of Adipose-Derived Mesenchymal Stem Cells. Mol Ther Nucleic Acids 2016;5:e285.

[143] Almalki SG, Llamas Valle Y, Agrawal DK. MMP-2 and MMP-14 Silencing Inhibits VEGFR2 Cleavage and Induces the Differentiation of Porcine Adipose-Derived Mesenchymal Stem Cells to Endothelial Cells. Stem Cells Transl Med 2017;6:1385-1398.

[144] Hamedi-Asl P, Halabian R, Bahmani P, Mohammadipour M, Mohammadzadeh M, Roushandeh AM, et al. Adenovirus-mediated expression of the HO-1 protein within MSCs decreased cytotoxicity and inhibited apoptosis induced by oxidative stresses. Cell Stress Chaperones 2012;17:181-190.

[145] Tsubokawa T, Yagi K, Nakanishi C, Zuka M, Nohara A, Ino H, et al. Impact of anti-apoptotic and anti-oxidative effects of bone marrow mesenchymal stem cells with

transient overexpression of heme oxygenase-1 on myocardial ischemia. Am J Physiol Heart Circ Physiol 2010;298:H1320-1329.

[146] Talele NP, Fradette J, Davies JE, Kapus A, Hinz B. Expression of alpha-Smooth Muscle Actin Determines the Fate of Mesenchymal Stromal Cells. Stem Cell Reports 2015;4:1016-1030.

[147] Lee PH, Tu CT, Hsiao CC, Tsai MS, Ho CM, Cheng NC, et al. Antifibrotic Activity of Human Placental Amnion Membrane-Derived CD34+ Mesenchymal Stem/Progenitor Cell Transplantation in Mice With Thioacetamide-Induced Liver Injury. Stem Cells Transl Med 2016;5:1473-1484.

[148] Latifi-Pupovci H, Kuci Z, Wehner S, Bonig H, Lieberz R, Klingebiel T, et al. In vitro migration and proliferation ("wound healing") potential of mesenchymal stromal cells generated from human CD271(+) bone marrow mononuclear cells. J Transl Med 2015;13:315.

[149] Yu Y, Liao L, Shao B, Su X, Shuai Y, Wang H, et al. Knockdown of MicroRNA Let-7a Improves the Functionality of Bone Marrow-Derived Mesenchymal Stem Cells in Immunotherapy. Mol Ther 2017;25:480-493.

[150] Zhang X, Huang W, Chen X, Lian Y, Wang J, Cai C, et al. CXCR5-Overexpressing Mesenchymal Stromal Cells Exhibit Enhanced Homing and Can Decrease Contact Hypersensitivity. Mol Ther 2017;25:1434-1447.

[151] Dong F, Patnaik S, Duan ZH, Kiedrowski M, Penn MS, Mayorga ME. A Novel Role for CAMKK1 in the Regulation of the Mesenchymal Stem Cell Secretome. Stem Cells Transl Med 2017;6:1759-1766.

[152] Zhang T, Li XH, Zhang DB, Liu XY, Zhao F, Lin XW, et al. Repression of COUP-TFI Improves Bone Marrow-Derived Mesenchymal Stem Cell Differentiation into Insulin-Producing Cells. Mol Ther Nucleic Acids 2017;8:220-231.

[153] Meng F, Rui Y, Xu L, Wan C, Jiang X, Li G. Aqp1 enhances migration of bone marrow mesenchymal stem cells through regulation of FAK and beta-catenin. Stem Cells Dev 2014;23:66-75.

Figure legends

Figure 1. Modes of MSC-based therapy.

Figure 2. Potential mechanisms of the MSC interactions with immune cells.

Figure 3. Schematic diagram illustrating the future of using modified MSCs for tissue/organ regeneration.

Table1. Clinical studies of MSC in chronic liver diseases

Study	Year	Design, F/U (month)	Patient cohort	Source of MSC	Injection route	Primary endpoint	Main improvement
Mohamadnejad et al. [94]	2007	Case series 12	Decompensated liver cirrhosis (n=4)	Autologous BM	Peripheral vein	Safety and feasibility	Creatinine and MELD score
Kharaziha et al. [92]	2009	Cohort 6	Liver cirrhosis (n=8)	Autologous BM	Portal vein (n=6) Peripheral vein (n=2)	Feasibility, safety, and efficacy (LFT and MELD score)	Creatinine, prothrombin time and MELD score
El-Ansary et al. [104]	2010	Case control 6	Decompensated liver cirrhosis due to HCV or HBV (n=12)	Autologous BM	Intra-splenic (n=6) Peripheral vein (n=6)	LFT and MELD score improvement	Creatinine, prothrombin time, albumin, bilirubin and MELD score
Amer et al. [97]	2011	Case control 6	Decompensated liver cirrhosis due to HCV (n=40)	Autologous BM	Intra-splenic (n=10) Intra-hepatic (n=10)	Safety and short-term efficacy (LFT, MELD improvement)	Ascites, peripheral oedema, albumin, MELD score, and Child-Pugh score
Peng et al. [99]	2011	Case control 1, 48	ACLF caused by HBV (n=158)	Autologous BM	Hepatic artery	Improvement of MELD and LFT (short term) or	Prothrombin time, albumin, bilirubin and MELD score

					development of HCC and mortality (long term)	
El-Ansary et al. 2012 [98]	Case control 6	Decompensated liver cirrhosis due to HCV (n=25)	Autologous BM	Peripheral vein	Improvement of MELD and LFT	Albumin and MELD score
Shi et al. [100] 2012	Case control 12 or 18	ACLF associated HBV (n=43)	Allogeneic UC	Peripheral vein	LFT and MELD improvement, adverse events, and survival rates	Albumin, prothrombin time, bilirubin, ALT, survival rates and MELD score
Zhang et al [16] 2012	Case control 12	Decompensated liver cirrhosis due to HBV (n=45)	Allogeneic UC	Peripheral vein	Safety and efficacy (LFT and MELD)	Albumin, bilirubin, MELD score and ascites
Amin et al. [90] 2013	Cohort 6	Post-HCV (n=20)	Autologous BM	Intra-splenic	Safety and efficacy	Albumin, prothrombin time, bilirubin, AST, ALT and MELD score
Mohamadnejad et al. [93] 2013	RCT 12	Decompensated liver cirrhosis (n=25)	Autologous BM	Peripheral vein	Safety and efficacy	None
Wang et al. [15] 2013	Cohort	UDCA-resistant PBC	Allogeneic	Peripheral vein	Safety and efficacy	Alkaline phosphatase and γ-

		12	(n=7)	UC			glutamyltransferase (GGT) levels
Jang et al. [91]	2014	Cohort 6	Alcohol related liver cirrhosis (n=11)	Autologous BM	Hepatic artery	Safety and efficacy	MELD score and liver histology
Salama et al. [95]	2014	RCT 6	Post-HCV end-stage liver disease (n=40)	Autologous BM	Peripheral vein	Safety and efficacy	MELD score and Child-Pugh score
Wang et al. [96]	2014	Cohort 12	UDCA-resistant PBC (n=10)	Allogeneic BM	Peripheral vein	Safety and efficacy	ALT, AST, GGT and IgM
Suk et al. [101]	2016	RCT 12	Alcohol related liver cirrhosis (n=72)	Autologous BM	Hepatic artery	Safety and efficacy	Histologic fibrosis and Child-Pugh score
Lanthier et al. [103]	2017	RCT 1	Decompensated alcoholic hepatitis (n=58)	Autologous BM	Hepatic artery	Safety and efficacy	None
Lin et al. [102]	2017	RCT 6	ACLF associated HBV (n=110)	Allogeneic BM	Peripheral vein	Safety and efficacy	Bilirubin, MELD score and survival rates

ACLF, acute-on-chronic liver failure; BM, bone marrow; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; LFT, liver function test; MELD, model for end-stage liver disease; PBC, primary biliary cholangitis; RCT, randomized controlled trial; UC, umbilical cord; UDCA, ursodeoxycholic acid

Table 2. Reported markers for selection and purification of MSC

MSC Source	Species	Markers expressed	Purification/ selection methods	Experimental Models	Target/ Mechanism	Ref.
BM	Human	$CD271^+$ and $CD56^+$	Cell Sorting	*In vitro*	• Increase clonogenic and proliferation potential. • Increase chondrocyte and pancreatic like cells differentiation.	[105]
BM	Rat	$CD73^+$	Cell Sorting	*In vitro* Lewis rats	• Enhance self-renewal and differentiation. • Increase engraftment.	[24]
BM	Human	$CD200^+$	Magnetic separation	*In vitro*	• Enhance regulation of bone resorption. • Inhibit osteoclast formation via inhibition of RANKL signaling pathway.	[112]
BM	Human	$CD200^+$	Cell Sorting	*In vitro*	• Suppress TNF-α secretion in macrophage like	[113]

					cells (Immunosuppressive activity)	
UP, AT, and BM	Human	αSMA+	FACS	*In vitro*	• Improve MSC fate through regulation of YAP/TAZ activation.	[146]
PAM	Human	CD34+	FACS	TAA (liver fibrosis model)	• Reduce hepatic fibrosis and restore liver function by reduce collagen level and deactivate the hepatic stellate cells.	[147]
BM	Human	CD271+	Magnetic separation	*In vitro* (model of wound healing)	• Significant potential in wound healing	[148]
UCB	Human	CD 146+	FACS	*In vitro*	• Reduce MSC senescence.	[107]
BM	Human	ALDH	FACS	*In vitro* and *in vivo* (NOD/SCID mice)	• Promote endothelial cell expansion. • Enhance recruitment of endogenous vascular cells in vivo by upregulation of lectin.	[114]

SYN and BM	Human	LNGFR and THY-1	FACS	*In vitro*	• Greater chondrogenic differentiation ability and colony forming potential than BM-MSC.	[110]
UC	Human	C362+ (Syndecan-2)	FACS	ALF	• Improve immunomodulatory properties and clonogenicity.	[119]
BM	Human	STRO-1	FACS	*In vitro*	• Increase expression of cardiovascular relate cytokines.	[116]

Bone Marrow (BM), Umbilical cord (UC), Umbilical cord blood (UCB), Synovium (SYN), Placenta amnion membrane (PAM), Adipose tissue (AT), Umbilical perivascular (UP), Aldehyde dehydrogenase (ALDH). Stromal Precursor antigen-1 (Stro-1), Acute liver failure (ALF), Receptor activator of nuclear factor kappa-B ligand (RANKL)

Table 3. Reported factors and their effect in priming of MSC for tissue repair.

MSC source	Molecule name	Time of treatment	Biological Function	Ref.
Human BM Human AT	IL-1β, IL-23, IL-6	96 hours	• Enhance secretion of TGF-β. • Reduce production of IL-4. • Exhibit significance multi-lineage. differentiation capacity.	[121]
Human BM	IL-1	24 hours	• Increase production of G-CSF. • Increase production of IL-10.	[122]
Human BM	IFN-γ and TNF-α	24 hours	• Enhance osteogenic formation via expression of ALP. • Increase expression of bone matrix proteins.	[124]
Human UC	IFN-γ, TGF-β, or multiple cytokine cocktail (IFN-γ, TGF-β, and retinoic acid)	72 hours	• Multiple cytokines cocktails improve the immunomodulatory properties of MSC. • TGF-β treated MSC increased recruitment of MSC to the liver injury *in-vivo*.	[119]
Mouse BM	IFN-γ + TNF-α with IL-17	12 hours	• Mediate liver injury through activation of iNOS.	[125]

Human BM	IL-17	24, 48, and 120 hours.	• Induction regulatory T cells. • Inhibition of Th1 cytokines. • Enhance production of IL-6.	[126]
Mouse BM	IL-6	24 hours	• Improve viability of hepatocytes treated with CCL_4. • Decreased expression of pro-apoptotic markers (BAX, caspase-3, and LDH). • Reduced liver fibrosis in vivo.	[127]
Mouse BM	IFN-γ or (TNF-α and IL-1)	Not sure	• Increase upregulation of ICAM and VCAM.	[128]
Mouse BM	(IFN-γ + TNF-α + IL-1α) or (IL-1β + IFN-γ)	24 hours	• Increase ability of MSC to inhibit T cell proliferations. • Enhance secretion of chemokines such as CXCL-9 and CXCL-10.	[40]
Mouse BM	CXCL9	30 minutes	• Ameliorate the adhesion of MSC to murine endothelial cells.	[130]

Table 4. Genetically modified MSC

MSC source	Example of associated genes	Condition	Viral vector	Representative biological activities	Ref.
Mouse BM	IGF-1 overexpression	Liver cirrhosis	Adenovirus	• Ameliorate liver fibrosis by significant reduction in α-SMA, collagen deposition, and TGF-β1.	[140]
Mouse BM	Let-7a Knockdown	IBD and GVHD	siRNA	• Significant improvement in both models, by suppress T cell proliferation (decreased in $CD3^+$), increase MCP-1 secretion, and enhancing expression of Fasl/Fas.	[149]
Human BM	CXCR5 overexpression	CHS	Lentiviral	• Increase migration and engraftment of MSC to the site of injury. • Enhance immunomodulatory effects of MSC *in vivo* through inhibit of T cell	[150]

				proliferation and supress production of IFN-γ and IL-17.	
Human BM	CXCR4 overexpression	ALF	Lentiviral	• Enhance migration and improve liver regeneration.	[144]
Rat BM	CXCR4 overexpression	Lung injury	Lentiviral	• Improve migration and suppress inflammation of lung tissue by upregulation of IL-10 and downregulation of TNF-α.	[145]
Rat AT	miR-27b overexpression	Partial hepatectomy	Micro RNA	• Enhance liver regeneration through reduction in ALT, TNF-α, and IL-6 in serum. • Reduce expression of TGF-β, MMP2, and MMP9.	[142]
Rat MB	CAMKK1 over expression	AMI	siRNA	• Reduce scar formation and improve cardiac function *in vivo*.	[151]

Porcine AT	MMP-2 and MMP-14 knockdown	In-vitro	siRNA	• Enhance differentiation of MSC into endothelia cells by production of PECAM and V-cadherin. • Increase the formation of capillary like cells and Sc-LDL uptake.	[143]
Human BM	HO-1 overexpression	In-vitro	Adenovirus	• Enhance MSC survival and resistant to oxidative stress. • Enhanced anti-apoptotic and anti-oxidative capabilities of MSC	[144]
Rat BM	HO-1 overexpression	MI	Plasmid	• Enhanced anti-apoptotic and anti-oxidative properties and improved angiogenesis level.	[145]
Human BM	HGF overexpression	Liver Fibrosis (MDN model)	Adenovirus	• Promote liver function and reduce liver fibrosis via significant reduction in TGF-β and PDGF-bb.	[141]

Mouse BM	COUP-TF1 knockdown	Streptozocin-induced diabetic mice	siRNA	• Increase ability of BM-MSC to differentiate into IPCs.	[152]
Rat BM	Aqp1 overexpression	Tibia fracture Model	Lentiviral	• Enhance MSC migration *in vitro* and *in vivo* through modulation expression of FAK and β-catenin.	[153]

Insulin growth factor like-1 (IGF-1), Inflammatory bowel disease (IBD), graft versus host disease (GVHD), Contact hypersensitivity (CHS), acute liver failure (ALF), amniotic fluid (AF), calcium/calmodulin-dependent protein kinase kinase-1 (CAMKK1), acute myocardial infarction (AMI), matrix metalloproteinases (MMPs), heme oxygenase-1 (HO-1), hepatocyte growth factor (HGF), Di-methylnitrosamine (DMN), platelet-derived growth factor-bb (PDGF-bb), chicken ovalbumin upstream promoter transcriptional factor I (COUP-TFI), Insulin producing cells (IPCs), Aquaporin 1 (Aqp1), focal adhesion kinase (FAK).

Figure 1: Modes of MSC-based therapy.

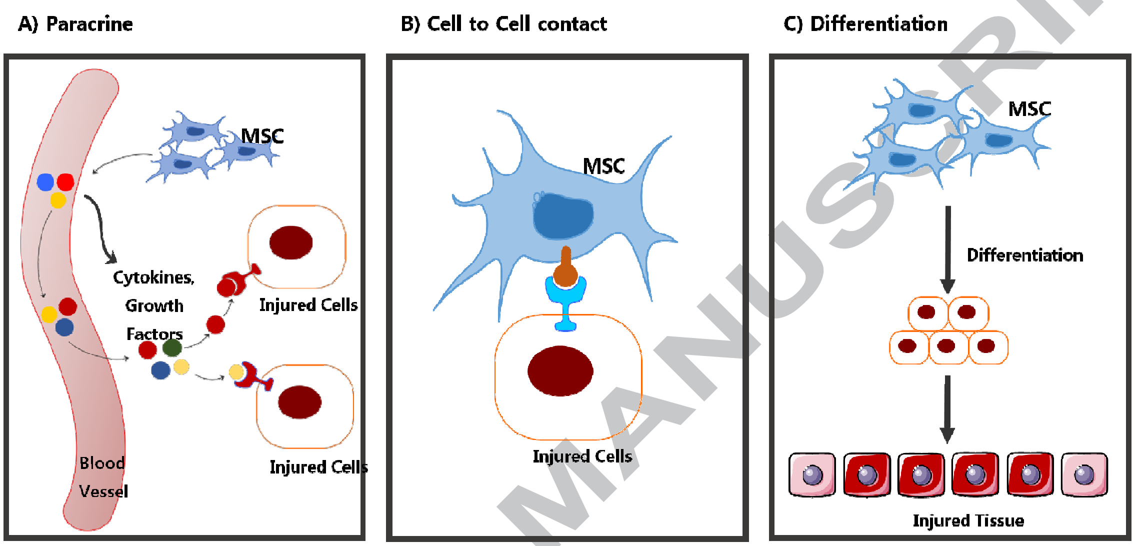
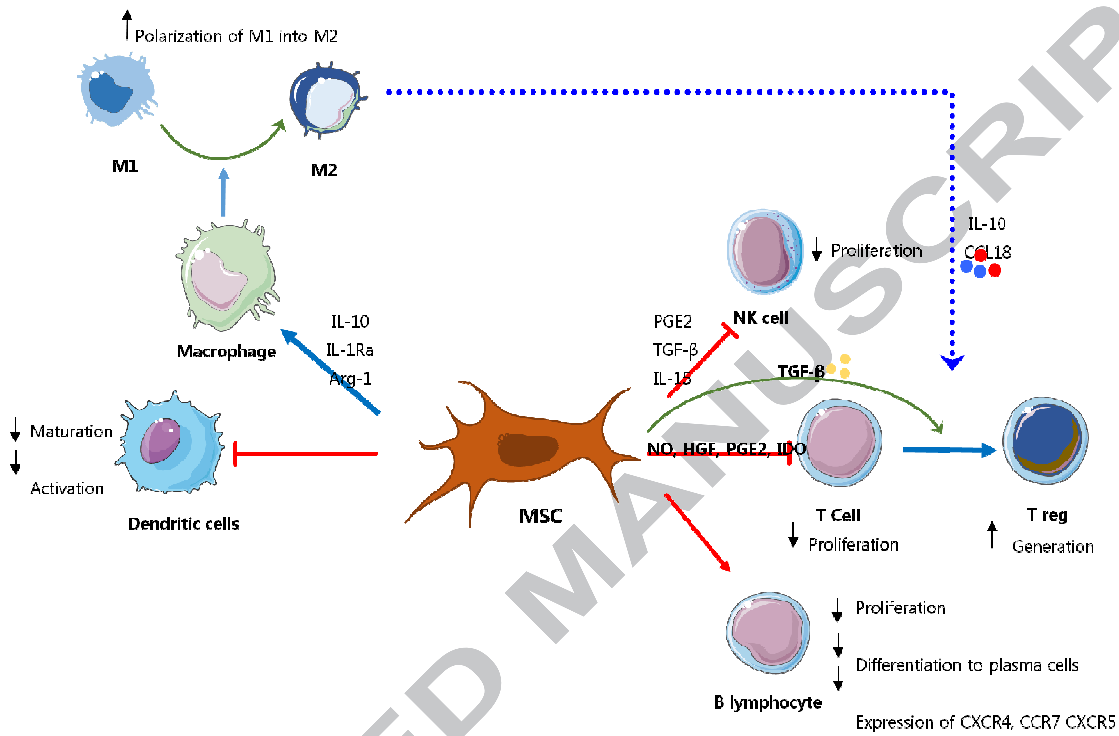


Figure 2: Potential mechanisms of the MSC interactions with immune cells



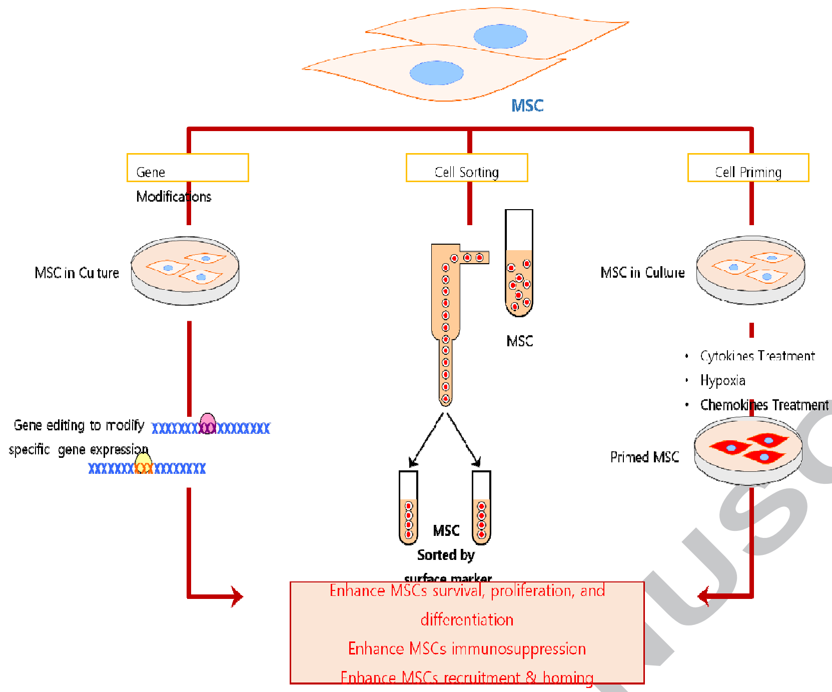


Figure 3: Schematic diagram illustrating the future of using modified MSCs for tissue/organ regeneration.